REMARKS

I. Preliminary Remarks

Applicants note, with gratitude, that many of the previous prior-art and formal rejections have now been withdrawn. Through the undersigned attorney, Applicants wish to thank the Examiner and her supervisor for the courtesy shown during the telephonic Interview conducted May 18, 2005 in which the remaining rejections were addressed. During that Interview distinctions between the steps of the claimed subject matter and the prior art were discussed and the Examiners asked several questions to which Applicants will respond below.

II. The Subject Matter of the Invention

The present invention is directed to methods for identifying intrabody frameworks that are stable and soluble in the intracellular environment. Previously, intrabodies were produced from monoclonal antibodies and were selected with classical techniques such as phage display. Although successful intrabodies were described it was unpredictable whether any particular intrabody would be functional within a cell. This did not relate so much to the binding affinity of the scFv for the target antigen but rather to the stability and solubility of the intrabody in the intracellular environment which was necessarily different from that in which they were produced. (e.g., phage display and other classical techniques were performed under oxidizing conditions while the intrabodies must function under reducing conditions.)

Nevertheless, because the solubility of an intrabody can be modified by either changes in the framework or the CDR's, there is value in determining whether the intrabody will be soluble and stable independent of the binding affinity of the scFv for an antigen.

The methods of claims 31, 35 and 36 are therefore based on the finding that the solubility/stability of a fusion protein comprising a marker protein and an intrabody is dependent on the solubility/stability of the intrabody moiety. Thus, if the intrabody moiety is soluble and stable in the intracellular environment then the marker protein can be detected and cells expressing such a stable fusion protein can be selected. These methods do not involve any interaction between a scFv and its corresponding antigen.

III. The Outstanding Rejections

Claims 31, 33-38 and 42-47 stand rejected under 35 U.S.C. §112 (first paragraph) for failure to comply with the written description requirement.

Claims 36-38 and 47 stand rejected as being anticipated under 35 U.S.C. §102(a) by Visintin et al. PNAS, 96(21):112723-11728 (October 12, 1999).

Claims 36-38 and 47 stand rejected as being anticipated under 35 U.S.C. §102(e) over Hoeffler et al. US 2003/0017149.

Claims 42 stands rejected under 35 U.S.C. §103(a) as being unpatentable over Visintin in view of Ptashne et al., US 20040014036.

Claims 31, 33 and 35 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Visintin.

Claim 34 stands rejected under 35 U.S.C. §103(a) as being unpatentable over Visintin in further view of Martineau, J. Mol. Biol. or Nolan et al. US 6,153,380.

Claims 31, 33-38 and 43-47 are provisionally rejected under the doctrine of obviousness-type double patenting over co-owned, copending application Serial No. 10/169,179.

IV. Patentability Arguments

A. The Rejections Under 35 U.S.C. §112 (First Paragraph) Should Be Withdrawn.

The written description rejections under 35 U.S.C. §112 (first paragraph) should be withdrawn because a review of the specification and figures make clear that the "quality control" assay of the present invention is carried out in a manner in which the detection of the marker protein is not dependent upon the presence of an antigen for which the intrabody is specific. An examination of Paras. 0073-0082 of the specification (quoted in part by the Examiner) describes the quality control process depicted in Figs. 1A and 1B and describes practice of the claimed assay in which intrabodies with "essentially identical antigen binding properties" were tested for in vitro solubility and stability. The specification teaches that "[o]nly two out of six tested scFv fragments [having identical antigen binding properties] were soluble and stable enough to activate reporter gene expression in our quality control system." Para 0082 Thus, the skilled reader would appreciate that the currently claimed method is present in the application as originally filed.

The rejections on the basis that it is "unpredictable whether an intrabody is functional within the cells" (Action, page 5) should be withdrawn because the claimed method is a screening assay the purpose of which is to determine that very functionality. Moreover, the determination of solubility and stability "in selected conditions" is likewise described and enabled. While it may be determined that an intrabody is unstable under a particular set of conditions, such a determination is not a failing of the assay but rather its purpose.

B. The Rejections of Claims 36-38 and 47 Under 35 U.S.C. §102 Should Be Withdrawn.

The rejections of independent claim 36 and claims 37, 38 and 47 depending therefrom over Visintin and Hoeffler should be withdrawn because both references rely upon the use of the two hybrid system for the isolation of intrabodies using an antibody/antigen interaction

wherein the claimed identification of the intrabodies is based on the interaction between the antibody and its corresponding antigen and does not determine whether an intrabody is soluble and stable as recited by claim 36. While the Visintin and Hoeffler assays will give a positive signal if an intrabody is soluble, stable and specific for the target antigen, they will not give a signal (they report a false negative) when the intrabody is soluble and stable but is not specific for (lacks binding affinity for) the target antigen. Attached hereto, is a copy of Exhibit A relating to this point which was used for illustration purposes during the Interview.

In the course of the Interview the undersigned attorney and the Examiners discussed the relevant disclosure of Applicants' specification directed to the "Quality Control" aspect of the invention which in the published specification comprises paras. 0073-0082. (page 16, line 24 through page 18, line 2 of the specification as originally filed). In discussing this disclosure, Supervisory Examiner Wang expressed a concern that contrary to the Applicants' arguments paragraphs 0073-0082 rely upon antibody-antigen binding and futher that the disclosure provides no examples in which a positive stability and solubility result is achieved in the absence of antigen-antibody binding.

As set out below, Applicants submit that the specification fails to imply that antigen-antibody binding is necessary to this aspect of the invention and further that it indeed provides an example scFv testing in which an scFv is identified having positive stability and solubility but lacking antigen binding affinity.

In response to the Examiner's concerns, it is pointed out that para. 0079 which concerned the Examiner discloses the interaction of the Gal11P-GalAD-scFv fusion protein with LexA-Gal4(58-97). This interaction is not an antibody-antigen interaction but rather is a two-hybrid reaction in which the "bait" is the Gal4 DNA binding domain (DBD) comprising LexA-Gal4(58-97) and the "prey" is the AD comprising the Gal11P-GalAD-scFv fusion protein.

Further, Applicants' disclosure illustrates examples of scFv's within each quadrant of the grid of Appendix A with the exception of scfVs which are neither soluble nor specific for the target antigen. In particular, the specification discloses an scFv which is soluble and stable but which does not specifically bind with the target antigen.

Submitted herewith as Appendix B is a further elaboration of the two-by-two grid of Appendix A comprising four quadrants. These quadrants are as follows: (a) including scFvs which are both stable and soluble for selected conditions and which are capable of specifically binding ("have a binding affinity for") a selected antigen; (b) scFvs which are stable and soluble and but which do not have a binding affinity for the selected antigen; (c) scFvs which are are not stable and soluble for selected conditions but which do have a binding affinity for the selected antigen; and (d) scFvs which are neither stable nor soluble and which do not have a binding affinity for the selected antigen.

As discussed at the Interview, the present invention is directed to a method for identifying intrabody frameworks and intrabodies which are stable and soluble and does not depend upon antibody-antigen binding to identify stable and soluble intrabodies. Thus, the method of the invention provides a positive signal indicative of a stable and soluble intrabody in both quadrants (a) and (b) while the prior art methods of Visintin, Hoeffler and others do not. (See Appendix A). Further, Applicants' specification discloses examples within each of quadrants (a), (b) and (c) illustrating this point.

Specifically, the specification at paras. 0073-0082 disclose the testing of six scFv fragments for solubility and stability. Several of those scFv fragments were also tested for binding affinity to a selected antigen (CGN4). The different scFv fragments having "essentially identical antigen binding properties but different in vitro stabilities" were expressed as Gal11P-4AD fusion proteins according to the method of the invention. (para.0078) Because, the intracellular stability and solubility of the Gal11P-Gal4AD-scFv

fusion proteins depended upon the scFv portion, only the stable and soluble scFv fusion proteins interacting sufficiently with the LexA-Gal4(58-97) "bait" were able to activate reporter gene expression. (Note again, the interaction between the Gal11P-Gal4AD-scFv fusion protein "prey" and the LexA-Gal4(58-97) "bait" is not an antibody-antigen binding interaction.)

The specification reports that "only two of six tested scFv fragments were soluble and stable enough to activate reporter gene expression..." (para. 0082) These two were (1) the "λ-graft" and (2) the "κ-graft." Other scFv fragments that were tested included (3) an anti-GCN4 wt scFv; (4) a destabilized point mutant [anti-GCN4(H-R66K)] scFv; and (5) a cysteine-free variant of the anti-GCN4 wild-type [anti-GCN4(SS^-)] As reported in para. 0082 these fusion proteins did not activate reporter gene expression in the claimed test system because they lacked stability and solubility. This lack of stability and solubility was confirmed in the fractionation analysis disclosed in Fig. 6. (see para. 0082)

The attention of the Examiners is also directed to the disclosure at paras. 0087-0089 and Table 1 in which the antibody-antigen binding affinity for the scFv fragments was measured in a direct binding assay which is different from the method claimed by Applicants. This testing reported low dissociation constants (Kds) for each of the λ-graft scFv, the anti-GCN4(H-R66K) scFv; and the anti-GCN4 wt scFv (low Kd equals high affinity) while the κ-graft scFv had low antigen binding affinity (despite having high stability and solubility). Arrangement of each anti-GCN4 scFv according to quadrant in Appendix B reveals quadrant (a) with the λ-graft; quadrant (b) with the κ-graft; quadrant (c) with each of anti-GCN4(H-R66K) scFv, the anti-GCN4(SS^-) scFv and the anti-CGN4wt scFv and quadrant (d) with no members. A comparison of these data points in Appendix B against the quadrants of Appendix A shows both that Applicants' method is different from that of the prior art and that it is described by the original application disclosure.

C. The Rejection of Claims 31, 33-35 and 42 Under 35 U.S.C. §103 Should Be Withdrawn.

Applicants note with gratitude the withdrawal of the various anticipation rejections of claims 31 and 33-38 over references such as Worn, Taliana and Visintin. These anticipation rejections under 35 U.S.C. §102 have now been replaced by obviousness rejections of claims 31, 33-35 and 42 under 35 U.S.C. §103 over Visintin either alone or in combination with other references. These rejections should also be withdrawn because not only does Visintin fail to disclose the detection methods of the application wherein the detection of the marker protein is not dependent upon the presence of an antigen for which the intrabody is specific but it would not have been obvious to modify Visintin in the manner claimed.

This is because Visintin Fig. 1 describes an "antibody-antigen ... interaction assay" and relies upon the occurrence of an antigen-antibody interaction to perform its assay. Thus, in the absence of an antigen-antibody interaction the Visintin assay would be are susceptible to providing falsely negative results regarding the stability of the intrabody framework because of the failure of antigen-antibody binding.

Specifically, Visintin relies upon the use of the two hybrid system for the isolation of intrabodies using an antibody/antigen interaction wherein the claimed identification of the intrabodies is based on the antigen dependent interaction between the antibody and its corresponding antigen. An examination of Figure 1 of Visintin et al. shows that the two-hybrid method was adapted "to detect antibody-antigen interaction in vivo." Thus, "[i]f antibody-antigen interaction occurs, in vivo, the resulting complex can bind to the LexA DBS upstream of his or lacZ genes" resulting in either growth of the transformed yeast or expression of a visible signal, respectively.) Again, a comparison of Figure 1 of Visintin with Applicants' Figure 1 is solicited.

The suggestion at pages 14 and 15 of the Office Action that Visintin's footnoted reference to Proba, J. Mol. Biol. would lead one to modify Visintin by testing for stability

prior to carrying out the two hybrid assay of Visintin is unsupported and unreasonable. First, Proba is only cited for the proposition "that some scFv have been shown to tolerate the absence of [a disulfide bond]. Moreover, Visintin itself teaches against Applicant's strategy and emphasizes that its strategy "would be to apply selection to the derivation of intracellular antigen-binding antibody fragments, which would allow isolation based on binding efficacy in vivo." The reference then further states that "[i]n these experiments, we have demonstrated that only certain antibody fragments can efficiently interact with their antigen in vivo." Page 11723, col. 2 (emphasis supplied)

The present invention is directed to methods for the identification of intrabody frameworks or intrabodies which are soluble and stable under selected conditions including reducing conditions. According to this method, the identification is not dependent upon any interaction between the antibody and its corresponding antigen. The rejections over each of the cited art references should be withdrawn because all those methods involve an interaction between the antibody and its corresponding epitope of the antigen.

Not only does Proba fail to correct the deficiencies of Visintin but so also do each of Ptashne, Martineau and Nolan which are directed only to specific aspects of dependent claims 34 and 42. For these reasons, the rejections under 35 U.S.C. §103(a) should be withdrawn.

D. The Provisional Obviousness-type Double Patenting Rejection Should be Deferred.

The provisional obviousness-type double patenting rejection over the related application should be deferred until an indication that the claims in both applications are otherwise allowable. At that time Applicants will consider the allowable claims of each case and either submission of arguments that the claims are unovbvious over each other or the submission of a terminal disclaimer.

CONCLUSION

For the foregoing reasons it is submitted that each of claims 31, 33-38 and 42-47 should now be allowed. Should the Examiner wish to discuss any issues of form or substance in order to expedite allowance of the pending application, she is invited to contact the undersigned at the number indicated below.

The Commissioner is authorized to charge any fee deficiency required by the paper to Deposit Account No. 13-2855.

Respectfully submitted,

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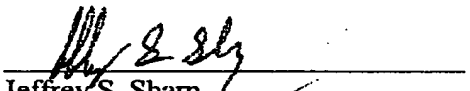
By: 

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